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**THE TONSILLAR CARRIAGE OF  
*YERSINIA* SPECIES BY PIGS**

**A THESIS PRESENTED IN PARTIAL FULFILMENT (40%)  
OF THE REQUIREMENTS FOR THE DEGREE OF MASTER  
OF PHILOSOPHY IN VETERINARY SCIENCE  
AT MASSEY UNIVERSITY**

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*"Let those who labour hold the reins"*

*M.R. Bishop*

*In memory of a Grenadian Hero and Martyr  
who endeavoured to uplift the standards  
of the working class through mass  
education and social reforms.*

*His spirit lives on!*

*To my family and children Maurice and Yenifer.*

## ABSTRACT

The impetus for this study arose due to the increasing isolation of species of *Yersinia* from people, with pigs being suspected as major reservoirs of human pathogenic strains of the organism in New Zealand. The general aims of the study, conducted in two phases, among pigs from several herds sent for slaughter at an abattoir in Palmerston North were:

- (i) to determine the presence of human pathogenic strains of *Yersinia* in the tonsils of slaughtered pigs and their distribution among selected herds,
- (ii) to determine the seasonal effect on prevalence of isolation and type of organism isolated, and
- (iii) to determine the *in vitro* virulence characteristics of strains of the organism isolated from the tonsils of slaughter pigs, and their potential public health implications.

The first phase involved a cross-sectional study, conducted between August and September, 1993. Tonsils were collected from 124 pigs from eight farms and were examined for the presence of species of *Yersinia*. A total of 77 (62.1%) strains of *Yersinia* were isolated, this consisted of 42 (33.9%), 27 (21.8%), 7 (5.6%) and 1 (0.8%) strains of *Y. enterocolitica*, *Y. pseudotuberculosis*, *Y. frederiksenii* and *Y. kristensenii* respectively. *Yersinia enterocolitica* serotypes 0:3, 0:5, 27 and *Y. pseudotuberculosis* comprised 26 (33.8%), 12 (15.6%) and 27 (35.1%) of the total number of isolates respectively. *Yersinia* were isolated from all eight farms with individual farm prevalences ranging from 20% to 100%, while the number of species per farm ranged from 1 to 3. The pyrazinamidase activity test correctly identified 48 of the isolates as pathogenic or non-pathogenic *Yersinia*, (a specificity of 96%).

The second phase, a longitudinal study, was conducted over a period of twelve months (February 1993 - January 1994), among pigs from four farms, selected according to the particular strain of *Yersinia* prevailing in the herd. A total of 705 pigs were examined for the carriage of species of *Yersinia* in their tonsils. A total of 264 isolates were obtained, consisting of 198 (75%), 55 (20.8%), 5 (1.9%), and 1 (0.4%) strains of *Y. enterocolitica*, *Y.*

*pseudotuberculosis*, *Y. intermedia*, *Y. frederiksenii* and *Y. kristensenii* respectively. *Yersinia enterocolitica* serotypes 0:5,27 and 0:3 comprised 105 (39.8%) and 78 (29.5%) of the total number of isolates respectively. *Yersinia pseudotuberculosis* comprised 55 (20.9%) with serotype III, 39 (14.8%) the most consistently isolated serotype.

*Yersinia* were isolated throughout the year particularly in the colder months. *Yersinia enterocolitica* serotypes 0:3 and 0:5,27 were found throughout the year with the lowest prevalence in the warmer months. However, a seasonal variation existed among serotypes of *Y. pseudotuberculosis*, with serotypes I and II found only in the winter and spring. Serotype III was found throughout the year, except for February.

During phase two of the study, 150 isolates of *Yersinia* were tested for *in vitro* virulence-associated characteristics. The autoagglutination test, CR-MOX agar, and the pyrazinamidase assay, coupled with salicin and aesculin tests, were highly successful in separating pathogenic from non-pathogenic strains of *Y. enterocolitica*. Likewise, the three assays successfully identified virulence activity in the majority of strains of *Y. pseudotuberculosis* with specificity among the three assays ranging between 90-100% for both *Y. pseudotuberculosis* and *Y. enterocolitica*.

The study also revealed marked variation in prevalence and type of *Yersinia* species isolated from pigs from different farms. The fact that particular serotypes predominate and persist on specific farms strongly suggest that there are factors such as source of pigs, management practices or contact with other animals which determine their status. Identification of these determinates could lead to control or eradication of important *Yersinia* from pig farms.

The overall prevalence of 41.1% ranks New Zealand among countries with reported high isolation rates of the organism and further emphasises the fact that pigs constitute major reservoirs for human pathogenic strains of *Yersinia* worldwide. The infection among slaughter pigs in New Zealand may be of human health concern and this warrants further investigation particularly to determine whether the strains isolated from pigs are identical to those involved in human disease.

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# TABLE OF CONTENTS

	Page
<b>ABSTRACT</b>	iii
<b>ACKNOWLEDGEMENTS</b>	v
<b>TABLE OF CONTENTS</b>	vii
<b>LIST OF FIGURES</b>	xi
<b>LIST OF TABLES</b>	xii
<b>LIST OF PLATES</b>	xv
 <b>GENERAL INTRODUCTION</b>	 xvi
 <b>CHAPTER ONE: REVIEW OF THE LITERATURE</b>	 1
<b>SECTION A: YERSINIOSIS</b>	1
<b>HISTORICAL AND GEOGRAPHICAL ASPECTS</b>	2
<b>GENERAL CHARACTERISTICS OF <i>YERSINIA</i> SPECIES</b>	6
The bacterium	6
Growth requirements	7
Isolation	8
Identification	9
Biotypes and serotypes	10
<b>EPIDEMIOLOGY</b>	13
Reservoirs	13
Transmission	14
Susceptible species	16
<b>PATHOGENESIS AND CLINICAL PRESENTATION</b>	17
<i>Yersinia pseudotuberculosis</i> infection	17
<i>Yersinia enterocolitica</i> infection	18
<b>YERSINIOSIS IN PIGS</b>	23
Infections with <i>Y. enterocolitica</i> , <i>Y. pseudotuberculosis</i> and <i>Y. pestis</i>	23
Anatomical distribution	27
Seasonal occurrence	29
<b><i>YERSINIA ENTEROCOLITICA</i> IN FOOD HYGIENE</b>	30



Occurrence in pork, related products and other foodstuffs	30
Foodborne outbreaks	22
<b>YERSINIOSIS IN NEW ZEALAND</b>	24
Human infections	24
Animal infections	34
<b>VIRULENCE ACTIVITY</b>	36
Pyrazinamidase Test	37
Calcium dependent growth at 37 °C	38
Congo Red uptake	38
Autoagglutination	38
<b>DIAGNOSTIC METHODS</b>	39
<b>TREATMENT, PREVENTION AND CONTROL</b>	41
Treatment	41
Prevention and control	42
<b>PUBLIC HEALTH IMPLICATIONS</b>	45
 <b>SECTION B: THE PORCINE PALATINE TONSILS</b>	47
INTRODUCTION	47
LOCATION AND ONTOGENESIS	47
CLASSIFICATION OF TONSILS	48
MICROSCOPIC ANATOMY	48
PATHOLOGY	49
 <b>CHAPTER TWO: CROSS-SECTIONAL STUDY OF THE OCCURRENCE OF <i>YERSINIA</i> SPECIES IN THE TONSILS OF SLAUGHTER PIGS IN THE MANAWATU</b>	54
INTRODUCTION	54
MATERIALS AND METHODS	55
Determination of sample sizes	55
Slaughter of pigs and sample collection	55
Processing of samples for cold-enrichment	56
Cultivation of species of cold-enriched samples	56

Screening for <i>Yersinia</i> species	57
Identification and serotyping of <i>Yersinia</i> isolates	57
Virulence assay	58
Biochemical media used	58
<b>RESULTS</b>	60
Occurrence and Distribution of <i>Yersinia</i> isolates	60
Biochemical and serological characterization of isolates	60
Pathogenic <i>Yersinia</i> isolates	61
Virulence assay	62
<b>DISCUSSION</b>	62
<b>CONCLUSIONS</b>	64

### **CHAPTER THREE: THE EFFECT OF SEASON ON PREVALENCE AND SPECIES OF *YERSINIA* ISOLATED FROM THE TONSILS OF SLAUGHTERED PIGS**

<b>INTRODUCTION</b>	66
<b>MATERIALS AND METHODS</b>	67
Farm selection	67
Determination of sample sizes	67
Animal reference	68
Sampling plan	68
<b>RESULTS</b>	68
Distribution and prevalence of species of <i>Yersinia</i>	72
Monthly isolation of yersiniae	72
<i>Yersinia enterocolitica</i>	72
Other <i>Yersinia</i> species	72
<i>Yersinia psuedotuberculosis</i>	74
Relationship between monthly isolation of Yersiniae and average daily temperatures	74
Distribution and seasonal incidence of species of <i>Yersinia</i> on individual farms	75
<b>DISCUSSION</b>	83

	x
CONCLUSIONS	86
CHAPTER FOUR: <i>IN VITRO</i> ASSESSMENT OF VIRULENCE AMONG STRAINS OF <i>YERSINIA ENTEROCOLITICA</i> AND <i>YERSINIA PSEUDOTUBERCULOSIS</i> ISOLATED FROM THE TONSILS OF SLAUGHTERED PIGS. PUBLIC HEALTH SIGNIFICANCE OF POTENTIAL PATHOGENS	88
INTRODUCTION	88
MATERIALS AND METHODS	90
Bacterial strains	90
Growth, isolation and identification procedures	90
Evaluation of sample tests used to define pathogenic serotypes of <i>Y. enterocolitica</i>	90
Temperature-dependent autoagglutination	91
Congo red, magnesium oxalate agar (CR-MOX)	92
Pyrazinamidase test	92
Salicin fermentation-aesculin hydrolysis	92
RESULTS	93
Virulence-associated characteristics in strains of <i>Y. enterocolitica</i>	93
Virulence-associated characteristics in strains of <i>Y. pseudotuberculosis</i>	94
DISCUSSION	94
CONCLUSIONS	98
CHAPTER FIVE: GENERAL DISCUSSION	99
RECOMMENDATIONS	103
APPENDICES	109
REFERENCES	135

## LIST OF FIGURES

	Page
Figure 1.1      The proposed route of infection for <i>Yersinia enterocolitica</i> .	15
Figure 1.2      Flow diagram for production and processing of pork products.	44
Figure 3.1      Individual farm prevalence of species of <i>Yersinia</i> isolated from the tonsils of pigs during the period February 1993-January 1994.	69
Figure 3.2      Relationship between monthly isolations of species of <i>Yersinia</i> and average daily temperatures.	76
Figure 3.3      Seasonal distribution and prevalence of <i>Y. pseudotuberculosis</i> serotype I from Farm K.	77
Figure 3.4      Seasonal distribution and prevalence of <i>Y. pseudotuberculosis</i> serotype II from Farm K.	77
Figure 3.5      Seasonal distribution and prevalence of <i>Y. pseudotuberculosis</i> serotype III from Farm K).	78
Figure 3.6      Seasonal distribution and prevalence of <i>Y. enterocolitica</i> serotype 0:3 from Farm T.	80
Figure 3.7      Seasonal distribution and prevalence of <i>Y. enterocolitica</i> serotype 0:5,27 from Farm W.	82

## LIST OF TABLES

Table 1.1	Interrelationships between serogroup, biovar, phagevar and geographical distribution of the most common human pathogenic strains of <i>Y. enterocolitica</i> .	5
Table 1.2	Relationship between biotype, serogroups, pathogenicity and geographic distribution of <i>Y. enterocolitica</i> .	6
Table 1.3	Biochemical differentiation of species within the genus <i>Yersinia</i> .	12
Table 1.4	Biochemical differentiation of <i>Y. enterocolitica</i> biogroups.	13
Table 1.5	Cardiovascular infections with <i>Y. enterocolitica</i> .	22
Table 1.6	The prevalence of <i>Yersinia enterocolitica</i> in pigs based on the findings of various investigators.	27
Table 1.7	Summary of some documented outbreaks of Yersiniosis.	33
Table 1.8	Methods used to define virulence in cultures of <i>Yersinia enterocolitica</i> .	37
Table 2.1	Flow diagram used in the isolation of <i>Yersinia</i> species from tonsils.	59
Table 2.2	Occurrence and distribution of <i>Yersinia</i> species from tonsils.	60
Table 2.3	Biochemical and serological characterization of 77 isolates of <i>Yersinia</i> .	61

Table 2.4	Prevalence of pathogenic <i>Yersinia</i> from the tonsils of pigs.	62
Table 2.5	Presence of pyrazinimide activity (PYZ) in the isolates of <i>Y. enterocolitica</i> and related species.	
Table 3.1	Farm distribution and prevalence of species of <i>Yersinia</i> isolated from the tonsils of pigs during the period February 1993 - January 1994.	70
Table 3.2	Species of <i>Yersinia</i> isolated in the study.	71
Table 3.3	Monthly isolation of species of <i>Yersinia</i> from the tonsils of pigs during the period February 1993 - January 1994.	73
Table 3.4	Serological characterization of 253 strains of <i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i> isolated during the study.	74
Table 3.5	Monthly isolation of species of <i>Yersinia</i> from Farm H.	75
Table 3.6	Monthly isolation of species of <i>Yersinia</i> other than <i>Y. pseudotuberculosis</i> from Farm K.	79
Table 3.7	Monthly isolation of species of <i>Yersinia</i> other than <i>Y. enterocolitica</i> serotype 0:3 from Farm T.	81
Table 3.8	Monthly isolation of species of <i>Yersinia</i> other than <i>Y. enterocolitica</i> serotype 0:5,27 from Farm W.	83
Table 4.1	Properties of <i>Y. enterocolitica</i> strains.	91

Table 4.2	Occurrence of virulence-associated characteristics among strains of <i>Yersinia enterocolitica</i> (Ye) isolated from the tonsils of pigs.	93
Table 4.3	Occurrence of virulence-associated characteristics among serotypes of <i>Yersinia pseudotuberculosis</i> isolated from the tonsils of pigs.	94

## LIST OF PLATES

Plate I	The palatine tonsils of the pig; paired lympho-epithelial organs.	52
Plate II	Histologic section of pig's tonsil, showing a tonsillar follicle, consisting of a crypt (C) with its associated lymphatic centres (L) H & E stain (x20).	52
Plate III	Histologic section of pig's tonsil, showing invagination of surface epithelium to form a crypt H & E stain (x40).	53
Plate IV	Histological tonsil of pig's tonsil, showing active lymphoid epithelium of a crypt H & E (x60).	53
Plate V	Porcine tongue, tonsils and pharynx. Location of tonsils are indicated.	65
Plate VI	Circumanal incision and removal of the intestines.	106
Plate VII	Excision of tongue, pharynx and particularly the tonsils.	106
Plate VIII	Post-mortem meat inspection involving incision of mandibular lymph nodes.	108
Plate IX	Deboning of head meat.	108



## GENERAL INTRODUCTION

Yersiniosis, a zoonotic disease caused by *Yersinia enterocolitica* and *Y. pseudotuberculosis*, is now recognised worldwide. The species *Y. enterocolitica* is an important cause of gastroenteritis in humans, especially in temperate countries (Mollaret *et.al.*, 1979; WHO, 1981, 1987). *Yersinia enterocolitica* is considered to be a foodborne pathogen, despite the fact that attempts to isolate the bacterium from foods implicated in cases of disease in humans have rarely proved successful. However, some large foodborne outbreaks caused by *Y. enterocolitica* have been reported in the U.S.A., Canada and Japan (see Table 1.7). Pork products are considered to be the most likely source of infection (Hurvell, 1981; Lee *et.al.*, 1981; Morris and Feeley, 1976), although some aspects of the epidemiology still remain to be clarified.

Pigs appear to constitute an important reservoir for *Y. enterocolitica* infection (Hurvell, 1981; Kapperud, 1991; Schiemann, 1989), and are the only food animal which regularly harbours pathogenic *Y. enterocolitica*. Pigs are often healthy carriers of *Y. enterocolitica* biotype 4/0:3 and biotype 2/0:9 strains which cause disease in humans. Biotype 1B/0:8, the predominant human pathogen in the U.S.A., appears to be rare in pigs. This serotype may have entirely different reservoirs and ecology (Schiemann, 1989). Serotype 0:3 and 0:9 are both faecal commensals and inhabitants of the oral cavity of pigs, especially the tonsils and tongues. Serotype 0:3 is also frequently encountered as a surface contaminant on freshly slaughtered pig carcasses (Andersen, 1988; Nesbakken, 1988; Nesbakken *et.al.*, 1985).

Many surveys, reviewed elsewhere (Table 1.6) have demonstrated the common occurrence of *Y. enterocolitica* and related microbes in the intestinal tract and oral cavity of healthy slaughter pigs. The earlier reports of *Y. enterocolitica* in pigs were based on examination of faeces or intestinal contents. It was later demonstrated that the isolation frequency of these bacteria was approximately ten times greater from the tongues or tonsils than that obtained from faeces (Pedersen, 1979; Schiemann, 1980; Wauters, 1979). The reported isolation rates range up to and in excess of 56.0% (Table 1.6) depending on the type of

samples examined (tongues, tonsils, throat swabs), geographical origin, and efficacy of the isolation methods.

In Belgium, which is the country with the highest reported incidence of *Y. enterocolitica* infection of people, a case control study has shown that the infection was strongly associated with eating raw pork (Tauxe *et.al.*, 1987). The apparent rareness of *Y. enterocolitica* infection in Moslem countries (Samadi *et.al.*, 1982) also supports the potential role of pork as the vehicle of *Y. enterocolitica* infection.

In New Zealand, reports of human disease due to yersiniosis, and of isolations from healthy subjects have been sporadic (Henshall, 1963; Lello and Lennon, 1992; Malpass, 1981; McCarthy and Fenwick, 1990; Rose, 1976), and an active search of possible hosts of species of *Yersinia* has only begun in earnest in recent years.

Prior to this study, only one published report of isolation of yersiniae from pigs in this country existed (Hodges *et.al.*, 1984). However, unpublished data by Fenwick (*pers.comm* 1989) suggested that pigs may be carriers of human pathogenic yersiniae in their tonsils.

This study was therefore conducted as a follow up to the former, with the aim of confirming the findings and establishing some epidemiological aspects of the occurrence of yersiniae in the tonsils of slaughtered pigs.

The study was conducted in two phases. The first phase involved a cross-sectional study to determine the presence of species of *Yersinia* in the tonsils of slaughtered pigs and their distribution among farms supplying pigs for slaughter. The second phase, a longitudinal study, which was based on findings from the first, involved a selection of farms in relation to their particular carriage of species of *Yersinia*. Abattoir sampling was carried out on a monthly basis for twelve months, with the objective of investigating the seasonal effects on the occurrence of species of *Yersinia* in the tonsils of slaughtered pigs. During this phase, isolates were tested for possible virulence-associated characteristics with the aim of determining the role of pigs as possible reservoirs for human infection with yersiniae and thus the potential public health significance of pathogenic strains which may be harboured as free-living commensals in the tonsils of slaughtered pigs.